

ATGGTGAATCGGTCGGTTGCGTTCTCGCGTTCGTTCTGATCCTTTTCGTGCTCGCCATC S < Þ S <

61 TCAGGTTATCAAATCTTTAGTTCATTTATTGAATATGATAGTATTTATATTCTTTTATGG

TTTTATGTGTTCTGACAAGTTGCAAATATTGAGTAGATATCGCATCCGTTAGTGGAGAAC intron <

181 TATGCGAGAAAGCTAGCAAGACATGGTCGGGAAACTGTGGCAATACGGGACATTGTGACA ス Þ ഗ ス 旪 Σ ഗ \bigcirc Z Н G 工

NCOI

ACCAATGTAAATCATGGGAGGGTGCGGCCCATGGAGCGTGTCATGTGCGTAACGGGAAAC Z S \Box Þ Þ G D \cap I < Z

301 ACATGTGTTTCTGTTACTTCAATTGTAAAAAAGCCGAAAAGCTTGCTCAAGACAAACTTA C 'n \bigcirc ĸ mj Z \bigcirc ス $\boldsymbol{\mathsf{x}}$ m HindIII スピ Þ 0 O ス

Z

361 AAGCCGAACAACTCGCTCAAGACAAACTTAATGCCCAAAAGCTTGACCGTGATGCCAAGA 0 0 0 罖 ٢ Z Þ 0 不 \Box

421 AAGTGGTTCCAAACGTTGAACATCCG Z < [1] 工 סי

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Fig. 1B.

GTGCCCCGGGTCACGAAGTTCGGCACATCTTAGCGTTATGCATAAGTCAAAAATGGCCAA Z Þ ス

61 AAATTCAGTTGCTTTCTTTGCATTGTGCCTGCTTCTTTCATTCTTGCTATCTCAGAAAT Z S < Þ т m Þ ٢ \cap ٣ D S **(T)**

121 CAGATCGGTGAAGGGGAATTATGTGAGAAGGCAAGCAAGACATGGTCTGGAAATTGTGG W ഗ < ス G m ۲ \cap [7] ス Þ S ス **-**3 Σ S 9 Z \cap 9

181 CAATACAAGACACTGTGATGACCAGTGCAAGTCTTGGGAGGGTGCAGCCCATGGAGCTTG 0 \circ $\boldsymbol{\varkappa}$ S $\mathbf{\Sigma}$ (7) GD D I G D \bigcirc

241 TCACGTGCGCGGTGGGAAACACATGTGCTTCTGCTACTTCAACTGTCCCAAAGCCCAGAA Ø 9 **(**) ス 二 3 \cap لب \cap ĸ m Z \bigcirc $\boldsymbol{\sigma}$ ス D 0 ス

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301 GTTGGCTGAGGATAAACTCAGAGCAGCAGAGCTAGCAAAGGAGAAGAATAATATTGGAGC F D m O ス \aleph Þ Þ (T) r D ス (T) ス Z Z Н S Þ

(1) ス < סי ഗ D \mapsto ď

361 TGAAAAGGTGCCTTCAGCCACACCTTGAGTACTAACAAA

Replacement Sheet

Fig.2A

Σ

GGCACGAGTAATGGCCAAAAATTCAGTTGCTTTCTTAGCATTTCTTGTGCTTCTT

M S × S S ω

61

S 幺 0 Ω

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ATGGTCTGGAAATTGTGGCAATACAAGACACTGTGATGACCAGTGCAAGTCTTGGGAGGG 121

CGCAGCCCATGGAGCTTGTCACGTGCGGGGGAAACACATGTGCTTTTGCTACTTCAA Σ I S G œ > H O Ø G I Ø Ø 181

CTGTTCCAAAGCCCAGAAGCTGGCTCAGGATAAACTCAAAGCCGACAAGCTCGCCAAGGA Ω O ¥ O

241

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GAAGAGTGAAGCCGAAAAGGTGCCAGCTACACCTTGAGTACTAACAAGTGTTGTATGATT 301

ATGAATAAAGAGAAAATGCTTTCTAGTTACCATATTTAGCATTCTCTAATGTGTAATGTT 361

TGTTGCTTTTGGAACTAATTGCTTAACTATGATTCCAGCTAATAATGTTTTAAGTATATA 481

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TGCTTCTTTTCGTTCTTGCTATCTCAGAAATTGGATCGGTGAAGGGAGAATTATGTGAGA

121 AGGCAAGCAAGACATGGTCTGGAAATTGTGGCATCACATCACACTGTGACAACCAGTGCC D S ス -Σ S S z G \vdash ഗ I \bigcirc a Z O \cap

GGTCGTGGGAGGGTGCAATCCATGGAGCTTGTCACGTGCGCGGTGGGAAACACATGTGCT I < × G **(**) ス 工 Z \bigcirc

TCTGCTACTTCAACTGTTCCAAAGCCGATGAGCTCGCGAAGGAGAAGATTGAAGCCGAAA K т Z \circ ഗ 大 D O m L D ス m [1] [1]

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301

361 GCTTTCTAAAAAAAAAAAAAAAAA

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61 CCTTTTCGTGCTCG¢CATCTCAGATATCACAAGTGTGAGAGGAGAAGTATGCGAGAAAGC ഗ ス -3 < Σ ഗ 9 Z, S 3 \bigcirc 0 < \bigcirc Z z $^{\infty}$ ---S ഗ \Box < < 工 Ø D G m < < \bigcirc mj m < ス \Box D

181 121 CTGGGAGGGGGGGGCCCATGGGGGGTGCCACGTGCGTGGAGGGAAACACATGTGTTTCTG TAGCAAGACATGGTCAGGAAACTGTGGCAACACGGGACACTGTGACAACCAATGTAAATA Σ ĸ nj (1) ス **(**) \cap D Þ 大 工 9 D \cap I < Ø G G \cap ス 0 工 Z \mathbf{z} 0 \cap \cap 'n 欠 \prec

241 CTACTTCAAGTGTCC¢AAAGCCGAAAAGCTTGCTCAAGACAAAGTTAATGCCCAAGAGCT m 不 Г D 0 Ö ス < Z 0 T Г

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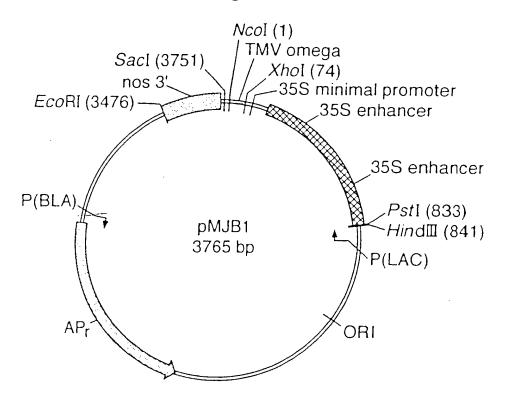
361 301 TTAAATAGAAAGTCTTAGATTACGAATGCGAATAACTATAGAAAATGTTTGCTAAATGTC TGACCGTGATGCCAAGAAAGTGATTCCGAACGTTGAACATCCGTGAAAGGGTCGGTTTCT

421 ACATTATAATTAGAACTTTATGATTGTTGTCAATAGGGCATTTTCTTGTTAGTGATATGT

481 GTAATAAGGTGATGCTTTTATGCTTTTCGTGCGTAAGAGTTTTCGACTATGTGTAATAAA

541 GAAAGGGTCTTTTTTTTTAAAAAAAAAAAAAAAAAA

Fig.4.



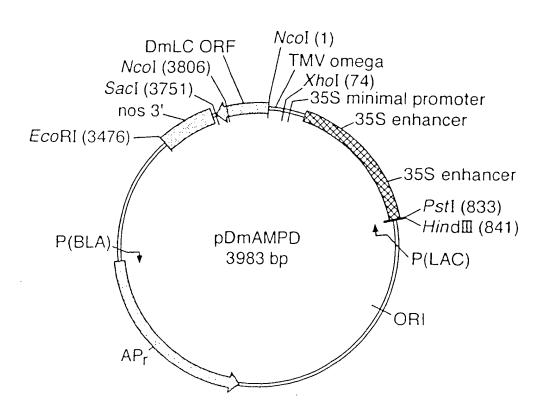
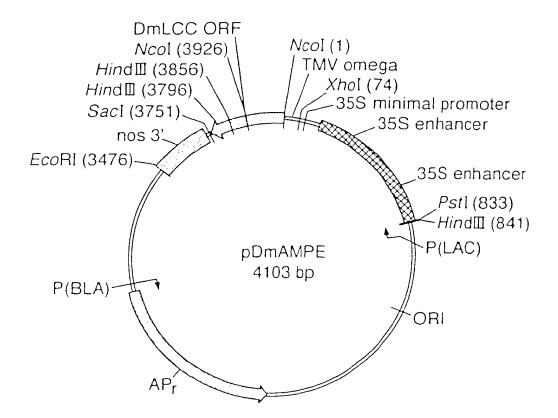
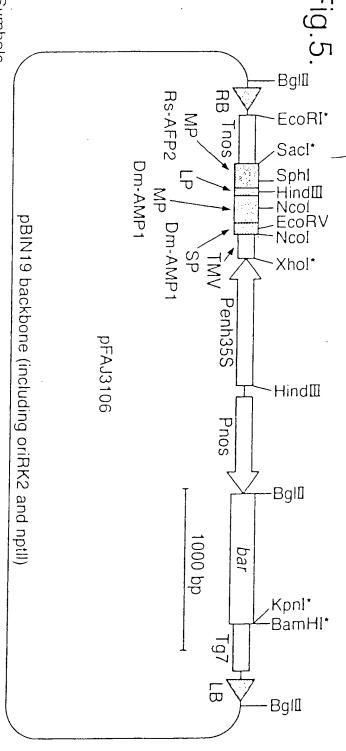


Fig.4 (Cont).





Symbols

RB: right border of T-DNA

Tnos: terminator of T-DNA nopaline synthase gene

LP: first 16 AA of Dm-AMP1 C-terminal propeptide and subtilisin-like protease recognition site IGKR MP Rs-AFP2: mature protein domain of Rs-AFP2 MP Dm-AMP1: mature protein domain of Dm-AMP1 cDNA

TMV: tobacco mosaic virus 5' leader sequence

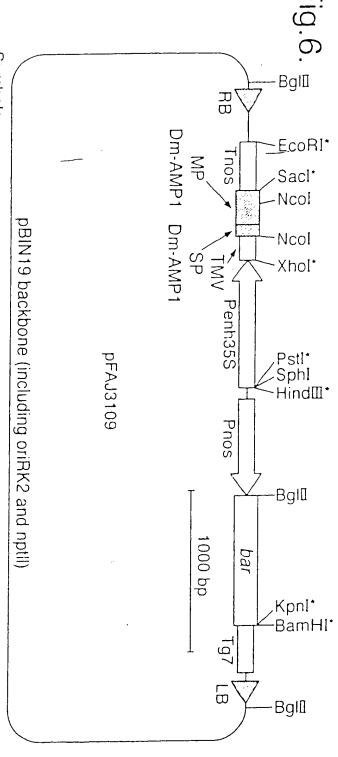
SP Dm-AMP1: signal peptide domain of Dm-AMP1 cDNA

Penh35S: promotor of 35S RNA of cauliflower mosaic virus with duplicated enhancer region Pnos: promotor of T-DNA nopaline synthase gene

bar: basta resistance encoding gene

Tg7: terminator of T-DNA gene 7
LB: left border of T-DNA

*: unique restriction site



Symbols

RB: right border of T-DNA

Tnos: terminator of T-DNA nopaline synthase gene

MP Dm-AMP1: mature protein domain of Dm-AMP1

SP Dm-AMP1: signal peptide domain of Dm-AMP1 cDNA

TMV: tobacco mosaic virus 5' leader sequence

Pnos: promotor of T-DNA nopaline synthase gene Penh35S: promotor of 35S RNA of cauliflower mosaic virus with duplicated enhancer region

bar: basta resistance encoding gene Tg7: terminator of T-DNA gene 7

_B: left border of T-DNA

unique restriction site

XhoI

NCOI

M V N R S V A F S A F V L I

 ${\tt TTCGTGCTCGCCATCTCAGATATCGCATCCGTTAGTGGAGAACTATGCGAGAAAGCTAGC}$ V L A I S D I A S V S G E L C E K A S

 $\mathtt{AAGACGTGGTCGGGCAACTGTGGCAACACGGGACATTGTGACAACCAATGTAAATCATGG}$

GAGGGTGCGGCÇCATGGAGCGTGTCATGTGCGTAACGGGAAACACATGTGTTTCTGTTAC KTWSGNCGNTGHCDNQCKSW

EGAAHGACHVRNGKHMCFCY

 ${ t TTCAATTGTAAAAAAGCCGAAAAGCTTGCTCAAGACAAACTTAAAGCCGAACAACTCATC}$ N C K K A E ·K L A Q D K L K A E Q L I

GGAAAGAGGCAGAAGTTGTGCCAAAGGCCAAGTGGGACATGGTCAGGAGTCTGTGGAAAC KRQKLCQRPSGTWSGVCG

AATAACGCATGCAAGAATCAGTGCATTAGACTTGAGAAAGCACGACATGGATCTTGCAAC D CKNQCIRLEKARHGSC

TATGTCTTCCCAGCTCACAAGTGTATCTGCTACTTTCCTTGTTAATAGGAGCTC P A H K C I C Y F

NcoI

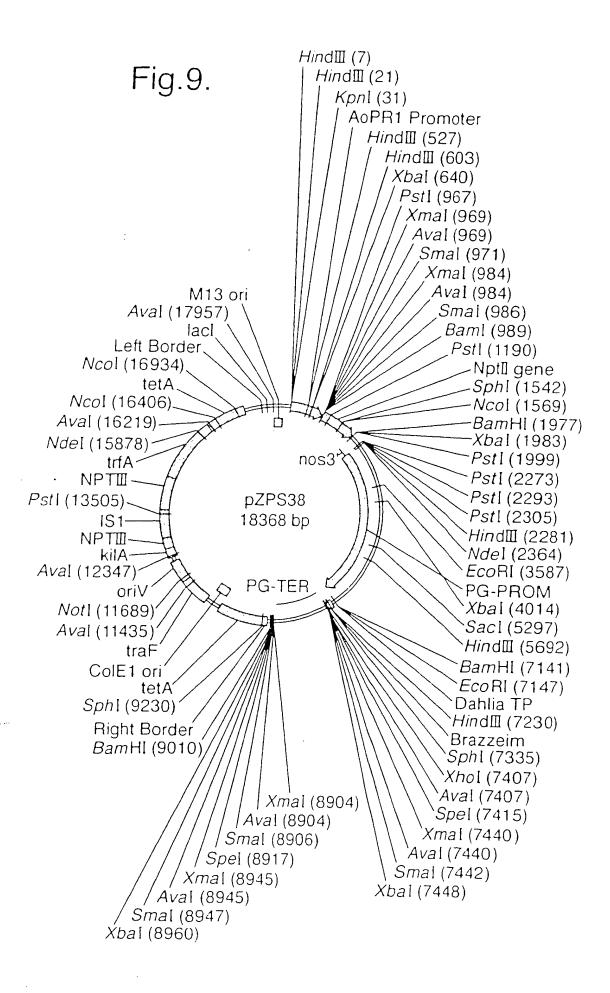
MVNRSVAFSAFVLIL

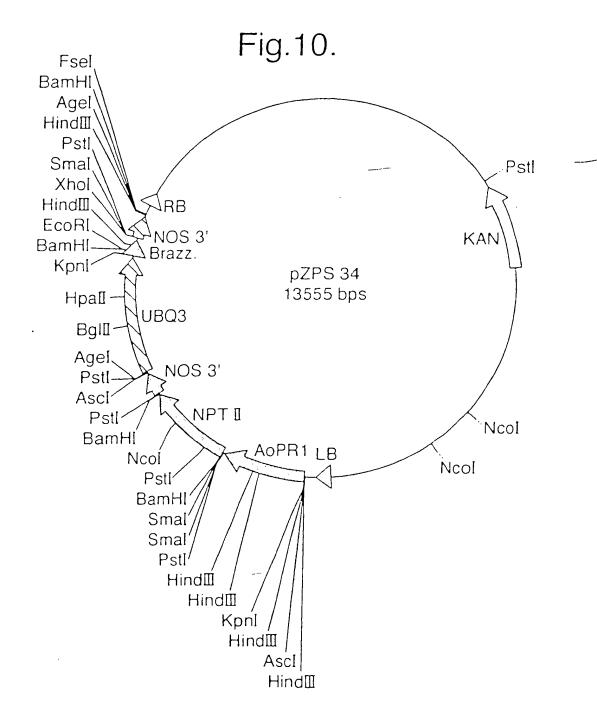
 ${\tt TTCGTGCTCGCCATCTCAGATATCGCATCCGTTAGTGGAGAACTATGCGAGAAAGCTAGC}$ V L A I S D I A S V S G E L C E K A S

 $\mathtt{AAGACGTGGTCGGGCAACTGTGGCAACACGGGACATTGTGACAACCAATGTAAATCATGG}$ TWSGNCGNTGHCDNQCKSW

GAGGGTGCGGCCCATGGAGCGTGTCATGTGCGTAATGGGAAACACATGTGTTTCTGTTAC NGKHMCFCY

TTCAATTGTTGAGCTC





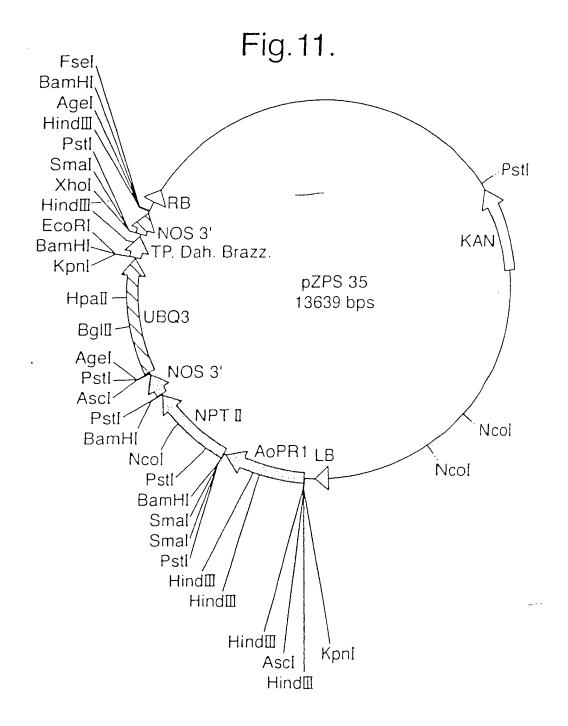


Fig. 12.

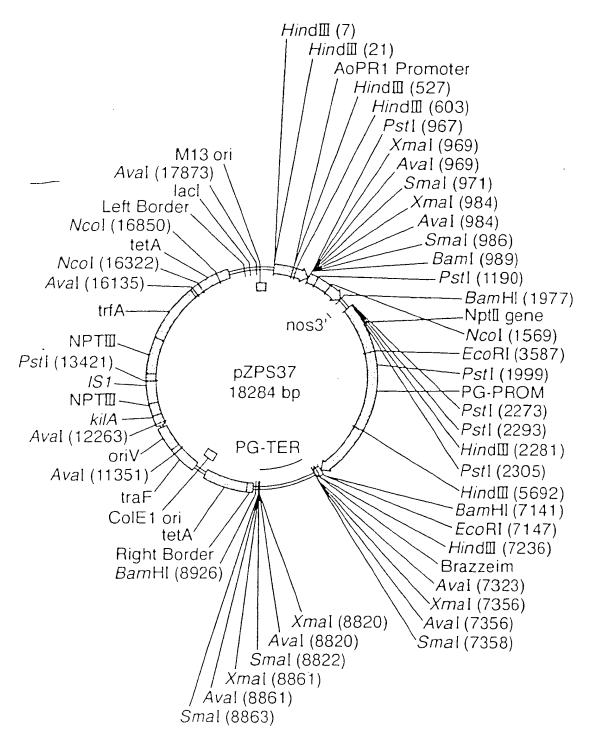
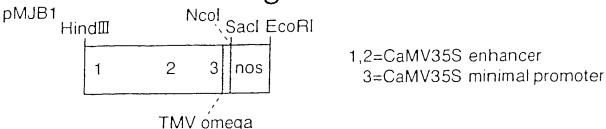
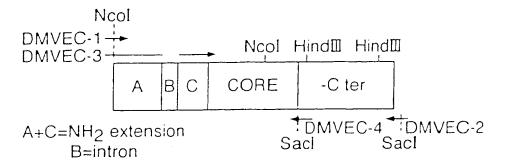


Fig. 13.



Structure of DmAMP1 Gene and position of vector construction oligonucleotides



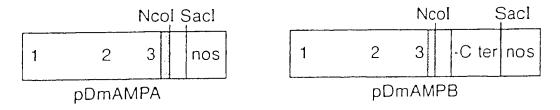
PCR Dahlia genomic DNA with DMVEC-1 and DMVEC-2, isolate 450 bp product.

PCR 450 bp DMVEC-1/DMVEC-2 PCR product with DMVEC 1 and 4.

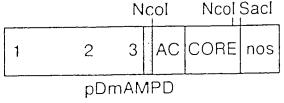
Isolate 60 bp Ncol / Sacl fragment, clone into pMJB1 Ncol / Sacl=pDmAMPA.

Cut 450 bp DMVEC-1/DMVEC-2 PCR product Ncol / Sacl . Isolate 180 bp Ncol /

Sacl fragment, clone into pMJB1 Ncol / Sacl =pDmAMPB



PCR 450 bp DMVEC-1/DMVEC-2 PCR product with DMVEC 3 and 4.
Isolate 150 bp Ncol fragment, clone into pDmAMPA
and pDmAMPB Ncol =pDmAMPD and pDmAMPE



		Nçol		Nc	ol S	Sacl	
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Fig.14.

Sequence ID No.6 Dm-AMP1

GAG CTT TGC GAG AAG GCT TCT AAG ACT TGG TCT GGA AAC

TGG GAG GGA GCT GCT CAT GGA GCT TGC CAT GTT AGA AAC

Sequence ID No.7 Dm-AMP2

GAG GTT TGC GAG AAG GCT TCT AAG ACT TGG TCT GGA AAC

Fig.14 (Cont).

TGC GGA AAC ACT GGA CAT TGC GAT AAC CAA TGC AAG TCT GGA AAG CAT ATG TGC TTC TGC TAC TTC AAC TGC

TGC GGA AAC ACT GGA CAT TGC

Fig.15.

